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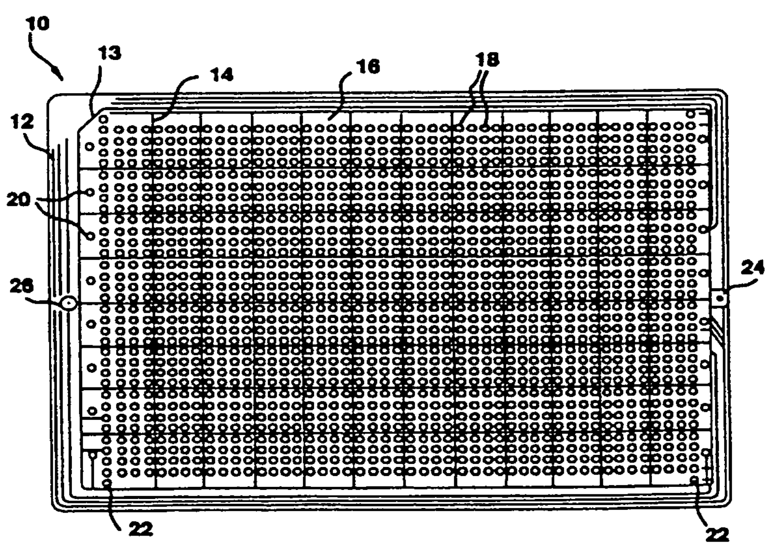

(57) Abstract

This application discloses a multi-well assay plate (10). The plate includes a peripheral skirt (12), a grid system (14), and an upper surface (16) having 1536 sample wells (18) arranged in a 128 x 192 row matrix, 16 control wells (20), and 4 calibration wells (22). The plate (10) is injection molded and has a matrix of wells arranged in mutually perpendicular rows. The plate (10) has a well density of greater than 1000 sample wells per plate.

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MULTI-WELL PLATE

Background

For many years, multi-well laboratory plates have been manufactured in configurations ranging from 1 well to 96 wells. The wells of multi-well plates are typically used as reaction vessels for performing various tests, growing tissue cultures, screening drugs, or performing analytical and diagnostic functions. Industry standard multi-well plates are laid out with 96 wells in an 8 x 12 matrix (mutually perpendicular 8 and 12 well rows). In addition, the height, length and width of the 96-well plates are standardized. This standardization has resulted in the development of a large array of auxiliary equipment specifically developed for 96-well formats. The equipment includes devices that load and unload precise volumes of liquid in multiples of 8, 12, or 96 wells at a time. In addition, equipment is available to transmit light through individual wells and to read colorimetric changes or chemiluminescence in individual wells. Some of this equipment is automated and instrumented to record, analyze and manipulate the data recorded. Recently, as sample sizes have been reduced to microliter levels and the demand for a greater number of tests per plate has

increased, the number of wells on a plate have likewise increased, e.g. from 384 wells to 1536 wells and above.

Summary of the Invention

It is therefore an object of the present invention to provide a multi-well plate that allows for handling sample sizes in the 1 microliter range. Further objects of the present invention are: to provide a multi-well plate with 1536 wells with incremental well spacing that is a fractionally based on the well spacing of the standard 96-well plate; to equip the plate with an additional 16 control wells and 4 calibration wells; to provide a plate capable of being sealed with heat sensitive or pressure sensitive film for controlling evaporation or long term storage; to provide a multi-well plate having wells with opaque side walls and transparent bottoms; to provide a two piece assembly multi-well plate that provides advantages in storage and ease of use; and to provide a method for producing the multi-well plate of the present invention.

Briefly, the present invention relates to an improved multi-well assay plate. Preferably, the plate has a matrix of 1536 wells, arranged in 48 columns and 32 rows. The plate is made of a thermoplastic material that is capable of being molded by injection molding. The dimensions of the plate conform to industry standards for a 96-well plate and the plate footprint is substantially identical to that of the 96-well plate. The plate additionally has 16 control wells and 4 calibration wells located on the periphery of the plate. The plate can be produced as a one piece molded assembly, or as a well matrix plate and frame comprising a two piece interlocking assembly.

Brief Description of the Figures

FIG. 1 is a plan view of the multi-well plate of the present invention.

FIG. 2 is a fractional cross-sectional view of two consecutive wells of the present invention.

FIG. 3 is a plan view of a well matrix insert of one embodiment of the present invention.

FIG. 4 is a plan view of the support frame of one embodiment of the present invention.

FIG. 5 is a cross-section view of a side wall of the frame of FIG. 4 taken along the section line 5-5 in FIG. 4.

FIG. 6 is a side view of the support frame.

FIG. 7 is a fractional cross-sectional view of a mold and ejection pins used in the molding process of the present invention.

Description of the Preferred Embodiments

Shown in FIG. 1 is a one-piece multi-well test plate **10** of the present invention. The plate includes a peripheral skirt **12**, a grid system **14**, and an upper surface **16** having 1536 sample wells **18**, 16 control wells **20**, and 4 calibration wells **22**. The sample wells **18** are preferably arranged in 48 columns spaced approximately 0.089 inches apart, measured from the center line of one column to the center line of the next consecutive column. Each column contains 32 wells. Sample wells in each column are likewise preferably spaced approximately 0.089 inches apart, measuring from the center of one well to the

center of the next well in the column. After every fourth column and every fourth well in each column is a grid line **14**. The grid lines are preferably grooves recessed from the surface of the plate and, when taken as a whole, divide the plate into 96 grids, each containing 16 sample wells. The grid system helps in identifying individual wells and locations that otherwise would be difficult to discern. The plate **10** preferably has a chamfered corner **13** which provides clear demarcation of the orientation of the plate.

Prior to the first column of sample wells and after the last column of sample wells are columns of control wells **20** having 8 wells each. The first control well is preferably displaced evenly between the second and third well of the nearest column of wells and approximately 0.089 inches away from the center line of the nearest column. The other seven consecutive control wells are preferably each displaced approximately 0.356 inches apart from the previous control well and approximately 0.089 inches away from the center line of the nearest column. A known amount of a known substance is retained in the control well. The control is used for analytical comparison to unknowns retained in the sample wells **18**.

A calibration well **22** is located at each end of the first and last columns. The calibration wells are preferably displaced approximately 0.089 inches from the next well in the column and are situated directly on the center line of the column. These calibration wells may retain a substance that is to be used in the calibration of certain analytical instrumentation. They can also be used for attaining proper alignment for particular assays.

The matrix of 1556 total wells (1536 sample wells **18**, 16 control wells **20**, and 4 calibration wells **22**) and surrounding area of plate surface is preferably raised

approximately 0.010 inches from the surrounding skirt surface **12**. This allows for coordinate lettering to be placed on the periphery or skirt **12** of the plate, while still maintaining a flat surface around the wells. A flat surface is important when sealing the wells with a pressure sensitive or heat sensitive film. Such sealing allows for control of evaporation and/or long term storage of compounds.

The one-piece multi-well plate **10** of the present invention has a hole **24** and slot **26** provided at opposite ends of the plate. The hole **24** and slot **26** are alignment features that allow the plate **10** to be positioned precisely on an analytical instrument or fluid dispenser, for example. Detents (not shown) may also be used to align the plate with a piece of instrumentation. Alignment pins, attached to auxiliary equipment, may be used to hold the plate in place. A pin placed through the hole accurately locates the plate, while a pin through the slot maintains parallelism while forgiving any length tolerance issues. The slot is critical because it allows for some dimensional flexibility in the finished part. The hole and slot features also aid in fluid transfer between first and second, substantially identical, plates. The slot **26** and hole **24** align one plate with an inverted twin plate. A second plate can be inverted and placed upon the first plate such that the slot from the first plate aligns with the hole from the second plate on one end, and the slot from the second plate aligns with the hole from the first plate on the opposite end. Any liquid sample contained in the inverted plate will remain in the wells due to surface tension. A pin can then be inserted through the aligned slot and hole on both ends of the plate, essentially locking them together. The upper surfaces of the two plates contact and individual wells

align such that, upon centrifugation, liquid in individual wells of one plate can be transferred to individual wells of a second plate.

The fluid transfer can be accomplished by using a standard 96-well centrifuge device having two vertical pins, corresponding to the hole **24** and slot **26** of the multi-well plate, and loading a first plate and an inverted second plate onto the pins. The plates are clamped in place using a spring clip on two or more sides and centrifuged.

Another interlocking embodiment (not shown) has an alignment pin situated on the surface of a first plate, capable of engaging a corresponding hole from a substantially identical and inverted second plate such that a pin from the first plate engages the hole from a second plate and the pin from the second plate engages the hole from the first plate.

FIG. 2 shows a cross-sectional view of two consecutive sample wells **18** of the present invention. The wells are cylindrical recesses in the surface **16** of the plate **10**. Each well has side walls **28** and a bottom wall **30**. The diameter of the wells at the surface of the plate is preferably approximately 0.059 inches. The diameter of the wells at the bottom of the well is preferably approximately 0.047 inches. Each well **18** is preferably approximately 0.060 inches deep, but may be deeper in order to position the sample therein closer to a detector located beneath the plate. Positioning the sample closer to the detector has the advantage of enhancing testing accuracy and minimizing crosstalk between adjacent wells.

The plate **10** is preferably made of a plastic such as polystyrene or polypropylene. The wells **18** preferably have opaque side walls **28** and a transparent bottom **30**.

The opaque side walls minimize crosstalk between wells. The clear bottom of each well allows colorimetric, fluorescent, or chemiluminescent testing to be performed from beneath the wells by standardized equipment. Well bottoms are preferably flat in order to enhance optical testing therethrough, but may be rounded, slanted or pointed.

FIGS. 3 and 4 are individual parts of a two piece construction that is an embodiment of the present invention. FIG. 3 shows a matrix well insert **32** that is used in conjunction with the frame **34** of FIG. 4 in forming a multi-well plate. The well matrix insert **32** preferably contains the same number of sample wells **18**, control wells **20**, and calibration wells **22** as the one piece construction of FIG. 1. Further, the spacing between wells is substantially identical to the spacing previously described for the one piece construction. A slot **26** and hole **24** are located on opposing sides of the insert in order to provide proper positioning on auxiliary equipment and to accommodate an inverted twin plate for purposes of liquid transfer between plates as described previously.

When dealing with an assay plate having wells of such small volume as the present invention (approximately 2 microliters), it is critical that the surface of the well plate remain flat. The well matrix insert **32** is flexible because it is thin. The well matrix insert preferably has a thickness of less than 0.200 inches, preferably about 0.100 inches, and preferably has a flatness of less than 0.015 inches.

FIGS. 4-6 show a frame **34** capable of receiving the well matrix insert **32**. The frame **34** is of a rectangular construction with four side walls **36** and is open through the center **38**. The outer dimensions of the frame (length, width and height) are approximately identical to the outer

surface dimensions of an industry standard 96-well plate. At least one of the side walls 36 has an opening or insertion region 33 through which the well matrix insert 32 can be inserted. The well matrix insert 32 slides through the frame 34 on tracks 35 in the side walls 36 until opposing detents in the form of depressions 40 located on the top surface of the well matrix insert and dimples (not shown) on the lower portion of the upper track on the frame 34, lock the insert and frame together. The frame 34 and well matrix insert 32 are further locked together by slots 42 in the well matrix insert and corresponding fitted extensions 44 on the frame. The insert 32 may also be tracked into the frame 34 in an inverted position. By inverting the insert in the frame, and subsequently inverting the entire assembly onto an optical reader, the well bottoms can be positioned closer to an optical reader. A chamfered corner 46 in the well matrix insert 32 allows for physical and visual orientation of the insert and the frame. When the well matrix insert is properly engaged in the frame, the resultant assay plate conforms to the industry standard and can be used with auxiliary equipment, including robots, designed for use with a standard 96-well plate.

The two piece construction embodiment of the present invention allows the well matrix insert to be removed from the frame and stored separately. Removing the well matrix insert reduces use of storage space by 60-80% over a one piece assembly or the interlocked two piece assembly.

Another embodiment comprises a two piece unit having an invertible well matrix insert and frame. In this embodiment, the frame is constructed such that the well matrix insert can be attached to a lower surface of the frame. The wells in the matrix insert retain the liquid

samples through surface tension. The inverted plate can then be aligned with optical sensors in the compatible instrumentation. The optical sensors that normally operate from below a multi-well plate and read color, fluorescence, or luminescence through optically transparent plastic of the well bottoms, can, in this embodiment, test the contents of each well through the well openings.

Additionally, an assembled two piece matrix plate or a one piece multi-well plate of the current invention can be inverted and placed on an optical sensing device. Surface tension will contain any sample fluid held in any well.

The injection molding method for forming the multi-well plate of the present invention involves a two stage ejection process. A plate having a plurality of wells comprising the well matrix previously disclosed, and as shown in FIG. 1, is injection molded. In FIG. 7, the surface of the mold **48** comprises a plurality of male well sections **50** that, when surrounded by injected plastic, create wells in the plastic. It is critical that the walls of the male well sections **50** have an inward slope of at least 3 degrees in order to ensure a molded plastic part **52** can be released from the mold. The mold itself has, within it, a series of knockout pins **54**,**56**. A portion of the wells are formed on the end of a first set of knock-out pins **54**. Such pins are preferably large enough to encompass a well or wells and ma_ come tangent to the next well edge, but do not include any part of the adjacent well. This provides for a knock out large enough to lift the molded plastic part **52** off the adjacent well molds without causing a bad steel condition in the mold, such as feather edges. A second set of knock out pins **56** are located on the periphery of the plate and do not

contact the wells at all. After molding, the first and second set of knock out pins **54** are extended, in order to lift the molded plastic part **52** off the core of the mold **48**. Next, the second set of knock out pins **56** located on the periphery of the mold are further extended in order to lift the molded plastic part **52** off the first set of pins **54**. This molding technique can be used for producing either the one piece multi-well plate or the well matrix insert. The frame used in the two piece construction multi-well plate is molded by conventional injection molding techniques.

Although preferred embodiments of the invention have been disclosed, other embodiments may be perceived without departing from the scope of the invention, as defined by the appended claims.

Claims

What is claimed is:

1. A multi-well assay plate comprising:

a well matrix having greater than 1000 sample wells;

said wells having sloped side walls and a substantially flat bottom;

said plate having length, width and height dimensions substantially identical to an industry standard 96-well plate; and

whereby said plate is formed using an injection molding technique.

2. The multi-well assay plate of claim 1 wherein said well matrix further comprises a plurality of control wells.

3. The multi-well assay plate of claim 1 wherein said well matrix further comprises a plurality of calibration wells.

4. The multi-well assay plate of claim 1 further comprising a pin, and a hole sized to receive a pin, located on opposing sides of said plate.

5. The multi-well assay plate of claim 1 further comprising a slot and a hole, sized to receive a pin on opposing sides of said plate.

6. The multi-well assay plate of claim 1 wherein said well matrix is comprised of 48 rows of wells having 32 wells in each said row.

7. The multi-well assay plate of claim 1 wherein said side walls are opaque and said bottoms are transparent.

8. The multi-well assay plate of claim 1 further comprising a series of grid lines whereby said lines divide said plate into defined grids.

9. The multi-well assay plate of claim 1 wherein said wells have a volume of no greater than 3 microliters.

10. The multi-well assay plate of claim 1 further comprising a chamfered corner.

11. The multi-well assay plate of claim 1 further comprising alignment detents.

12. A two piece assembly multi-well assay plate comprising:

an injection molded well insert having a matrix of sample wells;

said matrix having greater than 1000 wells;

said wells having sloped side walls and a substantially flat bottom;

said insert having an extended skirt region;

a rectangular frame unit having dimensions substantially identical to an industry standard 96-well plate;

said frame having side walls defining an open center region;

at least one of said walls of said frame having an insertion region capable of allowing ingress of said insert therethrough;

said side walls of said frame having tracks capable of receiving said extended skirt region of said insert; and

a means for locking said frame and said insert together.

13. The multi-well assay plate of claim 12 wherein said well insert further comprises a plurality of control wells.

14. The multi-well assay plate of claim 12 wherein said well insert further comprises a plurality of calibration wells.

15. The multi-well assay plate of claim 12 wherein said well insert further comprises a pin, and a hole sized to receive a pin, located on opposing sides of said well insert.

16. The multi-well assay plate of claim 12 wherein said matrix is comprised of 48 rows of wells having 32 wells in each said row.

17. The multi-well assay plate of claim 12 wherein said side walls of said wells are opaque and said bottoms are transparent.

18. The multi-well assay plate of claim 12 wherein said well insert further comprises a series of grid lines whereby said lines divide said well insert into defined grids.

19. The multi-well assay plate of claim 12 wherein said well insert has a chamfered corner.

20. The multi-well assay plate of claim 12 wherein said locking means comprises detents on said skirt of said well insert and on said frame.

21. The multi-well assay plate of claim 12 wherein said well insert has a thickness of less than 0.2 inches.

22. The multi-well assay plate of claim 12 wherein said well insert has a flatness of less than 0.015 inches.

23. A method for transferring the contents of one multi-well plate to another substantially identical multi-well plate comprising the steps of:

a) inverting a first multi-well plate having a defined well matrix and having a pin and hole on opposing ends, onto a second, substantially identical multi-well plate containing liquid sample, such that said pin of said first plate penetrates said hole of said second plate, and said pin of said second plate penetrates said hole of said first plate, and whereby said sample is retained within said second plate by surface tension;

b) applying centrifugal force to the interlocked plates in such a way that said liquid sample from said second plate is transferred to said first plate;

c) separating said first plate from said second plate.

24. A method for transferring the contents of one multi-well plate to another substantially identical multi-well plate comprising the steps of:

a) inverting a first multi-well plate having a defined well matrix and having a slot and hole on opposing ends, onto a second, substantially identical multi-well plate containing liquid sample, such that said slot of said first plate aligns with said hole of said second plate, and said slot of said second plate aligns with said hole of said first plate, thereby creating opposing hole-slot interfaces, and whereby said sample is retained within said second plate by surface tension;

b) threading a pin through said opposing hole-slot interfaces

c) applying centrifugal force to the interlocked plates in such a way that said liquid sample from said second plate is transferred to said first plate;

d) separating said first plate from said second plate.

FIG.1

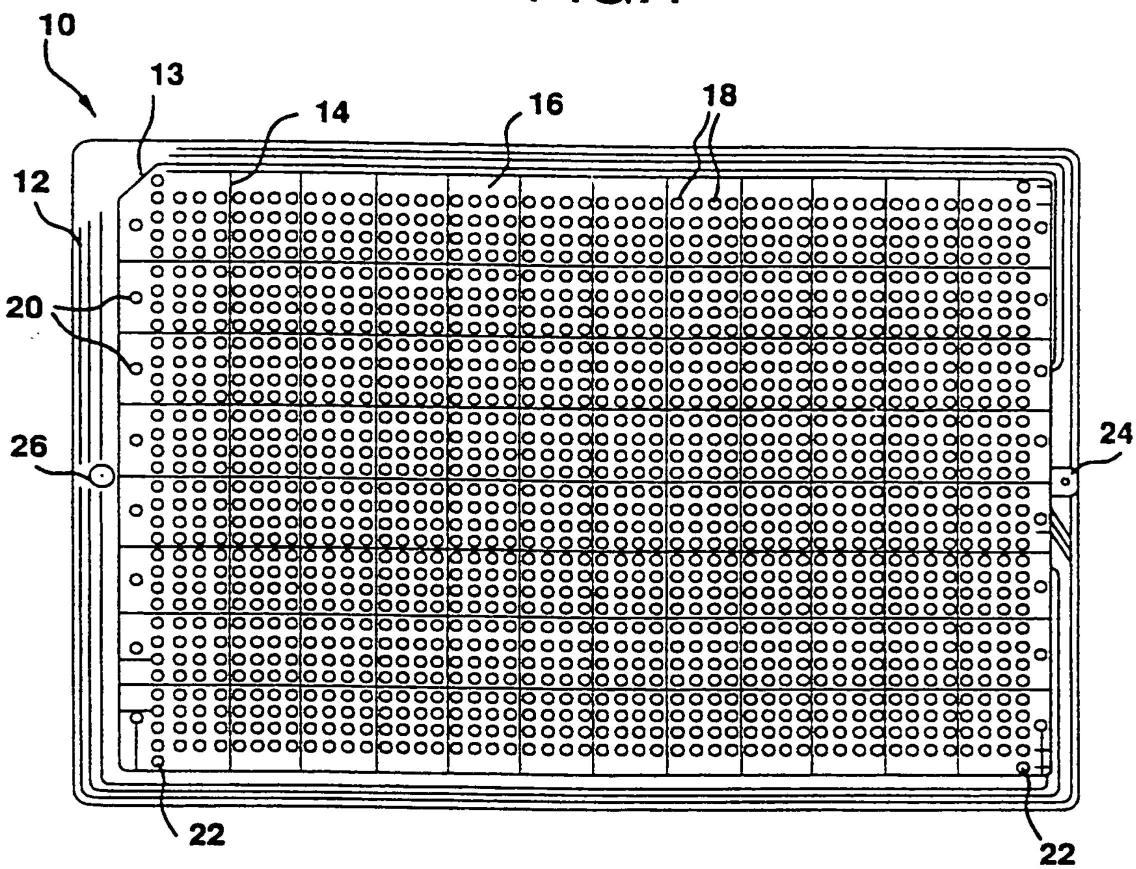


FIG.2

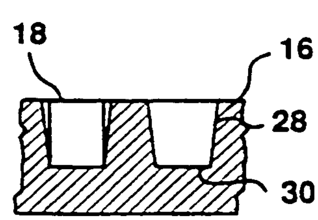


FIG.3

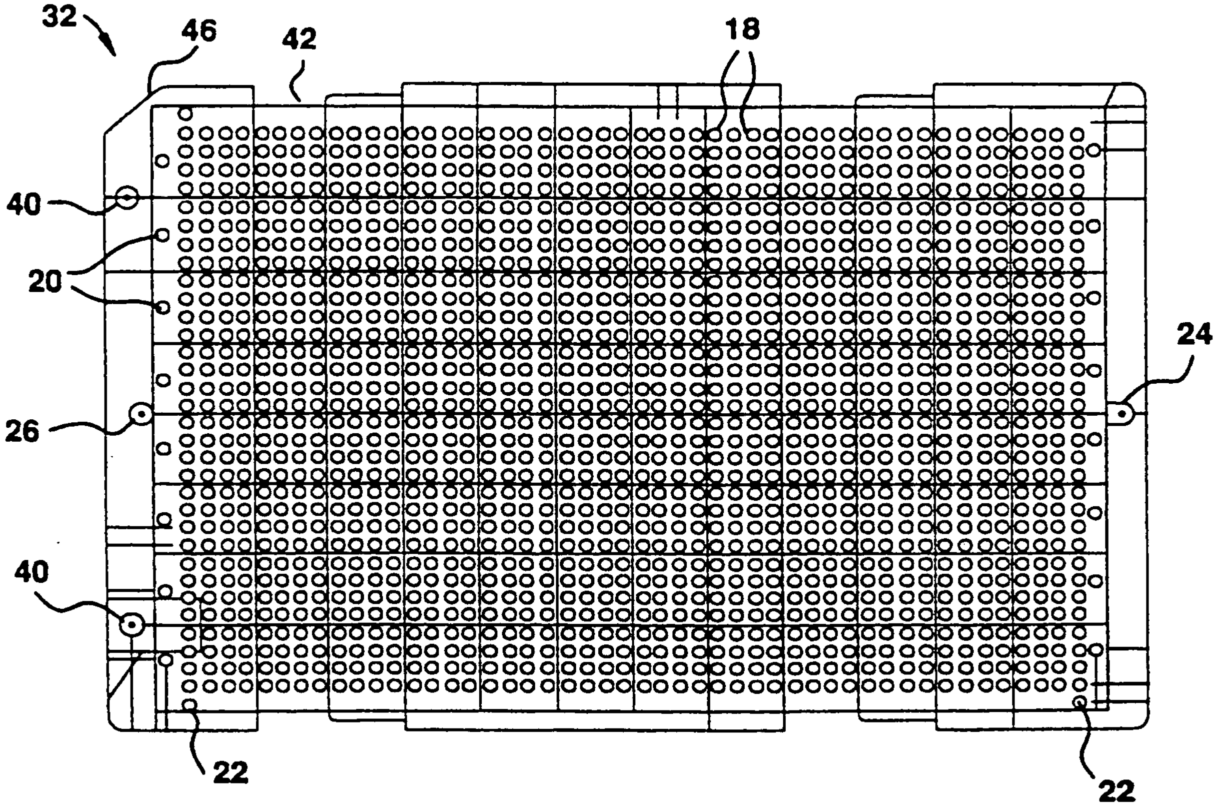


FIG.4

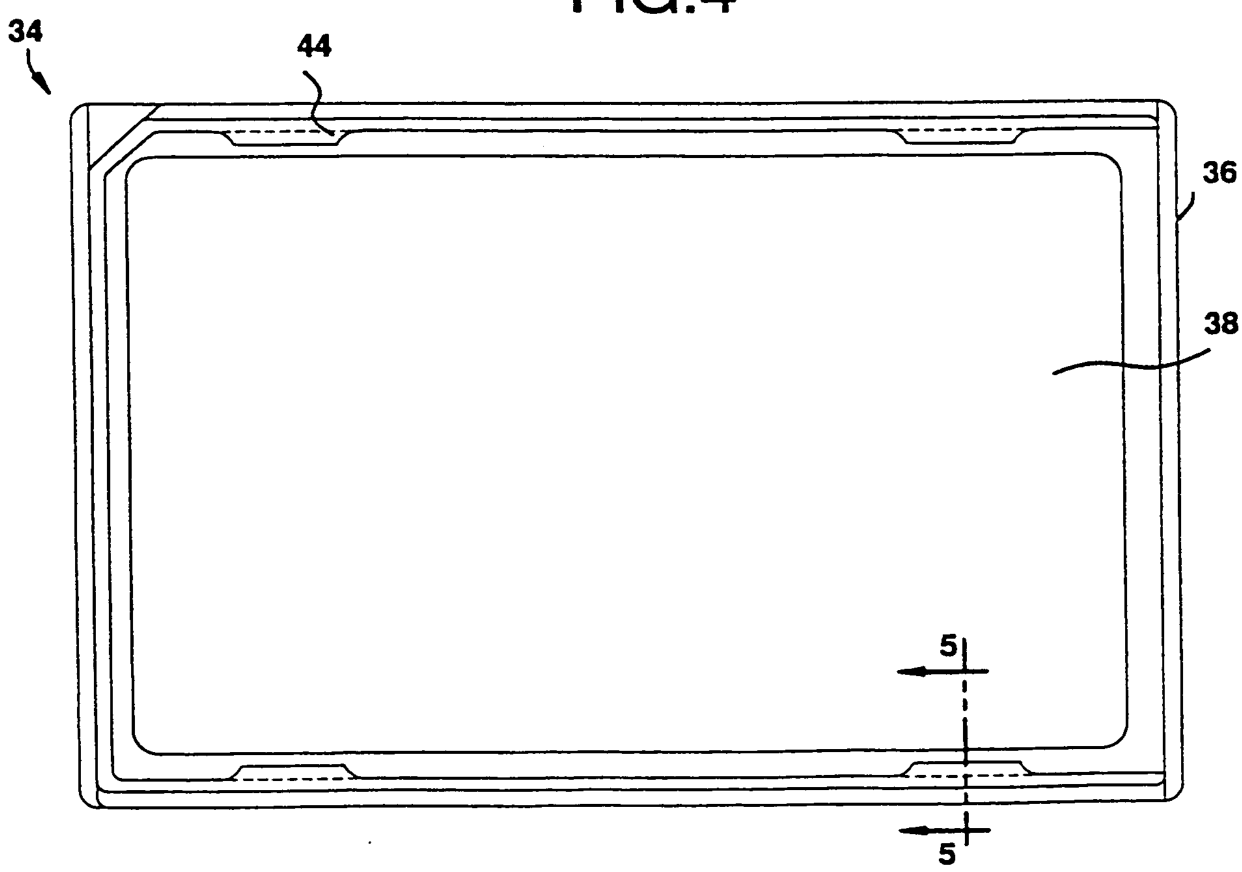


FIG.5

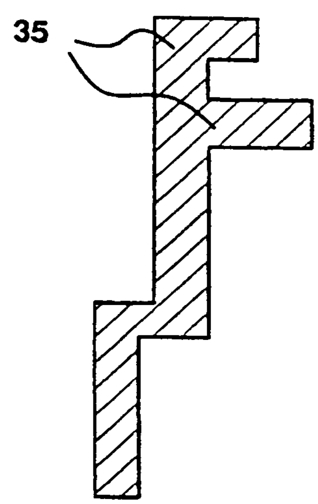


FIG.6

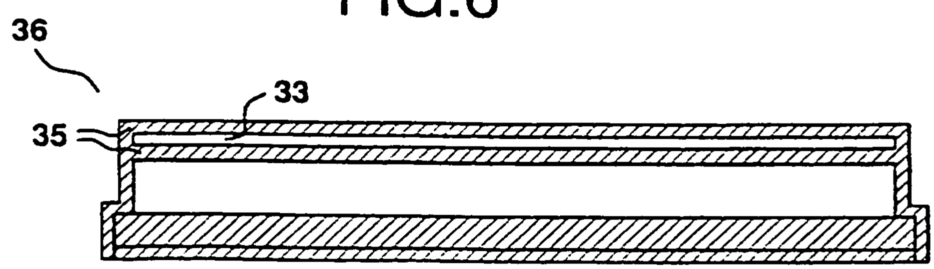
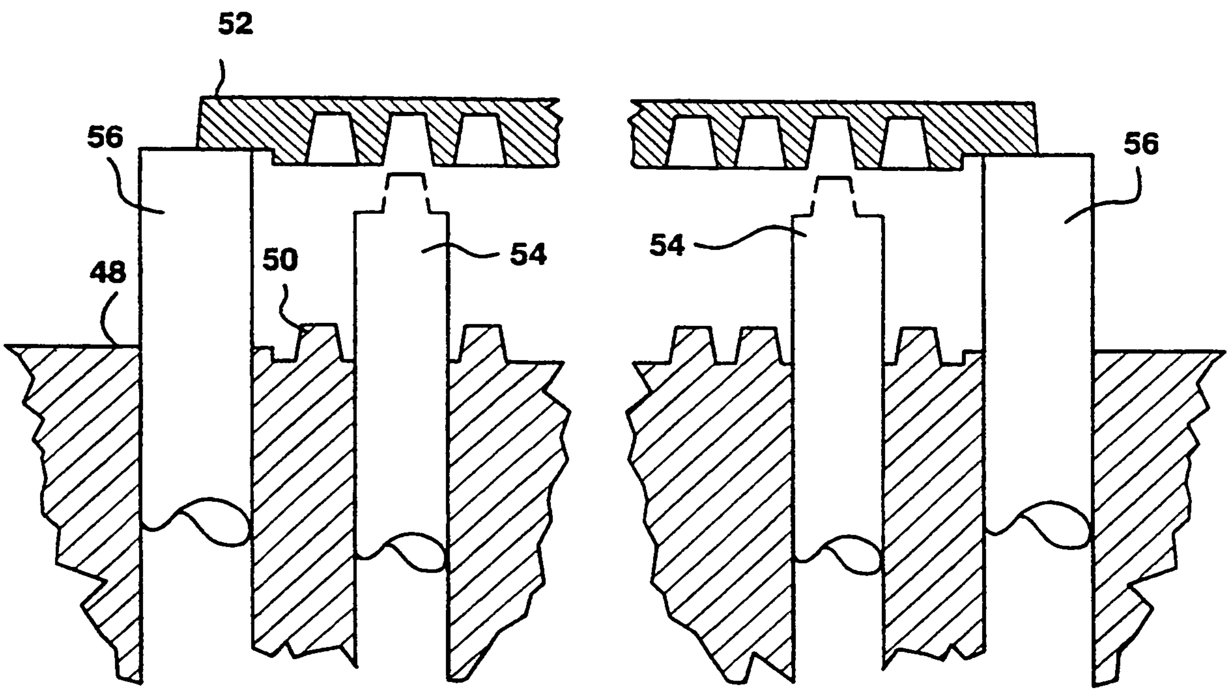


FIG.7



INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01L 11/00; C12M 1/12; C12M 1/20
US CL :422/101
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/101,102; 435/299, 300, 301, 311; 210/323.1, 955

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,294,795 A (LEHTINEN et al) 15 March 1994, see entire document.	1-24
Y	US 5,141,719 A (FERNWOOD et al) 25 August 1992, see entire document.	1-24
A	US 5,487,872 A (HAFEMAN et al) 30 January 1996, see entire document.	1-24
A	US 5,417,923 A (BOJANIC et al) 23 May 1995, see entire document.	1-24
A	US 5,039,493 A (OPRANDY) 13 August 1991, see entire document.	1-24
A	US 4,895,706 A (ROOT et al) 23 January 1990, see entire document.	1-24

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